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Catalytic Anions Embedded into Avidin: Importance of Their Chirality and the Chiral Environment on the Stereocontrol of the Aldol Reaction

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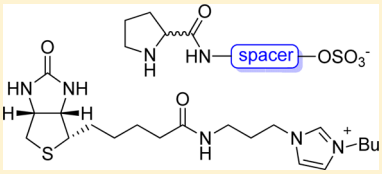
Supporting Information

ABSTRACT: Several catalytic anions bearing a pseudo-dipeptide scaffold, in combination with a biotinylated imidazolium cation, were prepared. The assembly of these salts with avidin resulted in the formation of stable biohybrid catalysts, active in ionic liquid/aqueous media for the aldol reaction. By using natural and non-natural amino alcohols as "side chains" for the proline derivative anion, we studied the cooperativity between the anion and its position in avidin. Taking advantage of the large freedom of movement of the anion inside avidin, we also investigated the substrate scope of this type of biohybrid catalyst.



INTRODUCTION

Over the last 50 years, enzymatic catalysis has proven itself to be a marvelous tool for synthetic chemists. Since the first discovery of enzymes almost two centuries ago, science has done much to unveil the mechanisms behind their incredible efficiency in doing specific chemical transformations and get a better insight of their structure−activity relationship.[1] The use of native enzymes in organic synthesis has been extensively studied over the past decades. Their field of applications ranges from kinetic resolution[2,3] to the introduction of specific functionalities on small molecules.[4] Enzymes are also tools of choice for asymmetric reactions, such as selective reductions, sometimes surpassing the best non-natural catalysts.[5] However, they suffer from major drawbacks. Among them, one can pinpoint a low substrate scope, a poor tolerance to common reaction conditions, and only a handful of possible tranformations. Directed evolution has been one solution to solve these issues, as native enzymes can be engineered to overcome their substrate specificity and gain robustness over somewhat harsh operating conditions.[1] More recently, the design of artificial enzymes has been another way to overcome many of these issues.[6] The design of these species relies mainly on the covalent or noncovalent incorporation of a small catalyst inside a biomolecule, such as a protein[7] or DNA.[8,9] These macromolecules provide a natural and complex chiral environment around the encapsulated catalyst, thus leading to the formation of a wide family of chiral biohybrid catalysts: namely, artificial enzyme mimics or non-native enzymes. Among several incorporation strategies, the pioneering work of Whitesides and Wilson consisted of using the biotin−avidin technology to anchor a biotinylated Rh complex inside avidin, leading to the formation of an artificial.hydrogenase.[10] This artifical metalloenzyme was used for the asymmetric reduction of *N*-acetylalanine. Over the past decade, Ward et al. focused a part of their research toward the development of numerous artificial metalloenzymes based on the biotin−(strept)avidin complex.[11,12] Ward et al. elegantly showed that chemical optimization of their ligands, combined with rational protein engineering, can lead to the design of efficient artificial enzymes tailored for specific reactions.[13,14] Our group recently disclosed the conception and design of biohybrid species by encapsulating inside avidin a biotinylated imidazolium salt bearing a nonchiral pyrrolidine-based catalytic anion. The assembled biohybrid catalyst was proven to be active in ionic liquid/water mixtures, efficiently catalyzing asymmetric aldol reactions.[15] While the reaction medium was shown to have a great influence on the behavior of our catalyst, very little change was observed when modifying the structure of the cationic core of the imidazolium salt (Figure 1).

In order to gain a better understanding of how the anion positions itself inside avidin, we prepared several proline-based anions and combined them with the same biotinylated imidazolium salt. The role of these anions in catalysis and the tolerance of the optimized system to other substrates are reported here.

RESULTS AND DISCUSSION

A total of eight anions were prepared, each displaying different physical and chemical properties. Our strategy was to keep the pyrrolidine moiety, responsible for catalysis, and insert a spacer between the amine site and the sulfate anion. We hypothesized that (1) adding an alkyl spacer might give flexibility to the anion and the length of this spacer might move the amine moiety apart from the imidazolium cation, placing the anion in a new chiral environment, (2) using chiral amino alcohols as spacers might be beneficial due to their intrinsic chirality, and (3) using specific amino alcohols might be interesting by providing the anion with specific properties, such as steric

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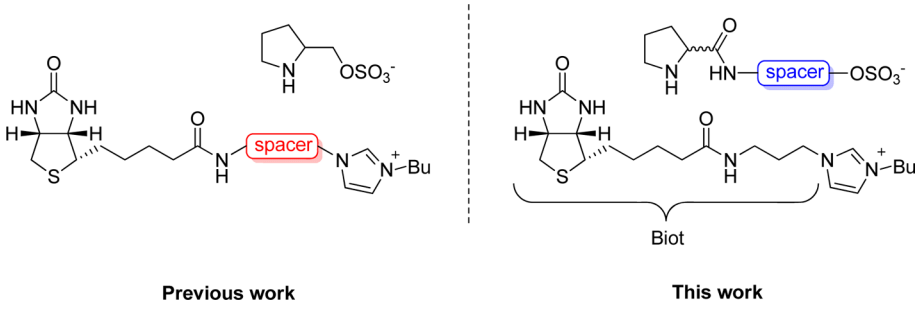


Figure 1. Modification of the anion structure.

bulkiness, conformational freedom, or $\pi-\pi$ interaction properties. In this way, the anion could interact with specific residues inside the avidin's cavity, leading to new conformations and positions when it is embedded in the protein (Figure 2).

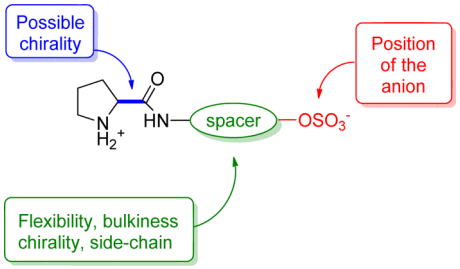


Figure 2. Design of the anions.

The synthetic strategy is described in Scheme 1: the desired chiral/achiral version of proline was first Boc-protected, and then a standard peptidic coupling between Boc-Proline **1** and the corresponding amino alcohol afforded the hydroxy precursor **2**, which was then sulfonated using chlorosulfonic acid in CH_2Cl_2. Amino alcohols were obtained from the corresponding amino acids following the procedure reported by Meyers et al. (see the Supporting Information).[16] Preparation of the corresponding biotinylated catalysts and encapsulation into avidin were carried out using our previously reported procedures.

All eight anion structures are reported in Figure 3, and the results obtained by using them in the aldol reaction are reported in Table 1. Anion **4**, the one used in our previous studies (Table 1, entry 1), was used as a means of comparison with the second generation of the anions.

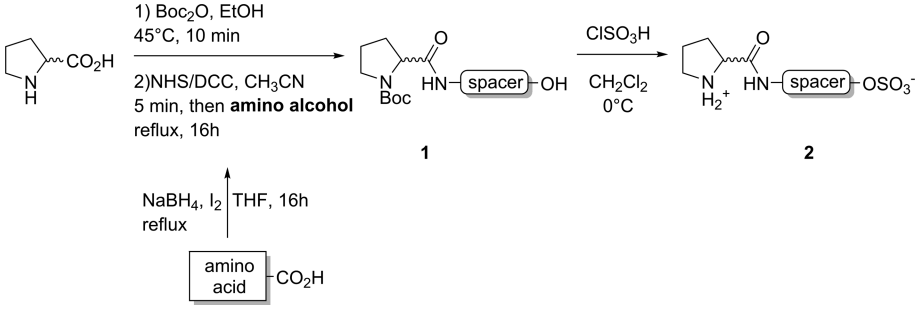
Each corresponding biotinylated catalyst was embedded into avidin and tested under our optimized reaction conditions, using a partitioned IL/aqueous buffer media. As control experiments, aldol reactions without avidin were carried out, with a substrate concentration 20 times higher in order to overcome the high dilution conditions necessary to ensure avidin solubilization ([substrate] = 5×10^{-2} M).[15] Cyclohexanone and *p*-nitrobenzaldehyde were the model substrates used for these studies. From the results compiled in Table 1, several observations can be drawn.

(a) The use of racemic anions **4** and **5** possessing superior flexibility led to a lower stereocontrol of the corresponding biohybrid catalyst (the notation ***X*·Av** will be used in this paper to design the biotinylated catalyst obtained by the complexation of avidin with the imidazolium cation bearing the anion ***X***), in comparison to our previously reported **3·Av**. When the corresponding biotinylated catalysts were not embedded into avidin, no enantioselectivity was observed. It seems that too much freedom in the avidin results in less steric discrimination, perhaps due to the fact that the anion can occupy several places inside the protein. Conversions in both cases (with and without avidin) are very similar to those we already reported for the aldol reaction.[17]

(b) When L-proline was used as the amine precursor (entry 4), a rise in selectivity up to 70% ee was observed for anion **6**, in comparison to the D,L-proline used in the case of anions **4** and **5**. This might be a hint of a match−mismatch case between avidin and the catalyst. More surprisingly in this case, when the reaction was carried out without avidin, the selectivity was the same.

(c) With L-Proline kept as a precursor, adding a chiral side chain derived from natural amino acids (L-phenylalanine for compound **7** and L-valine for compound **8**) led to better results in terms of selectivity. However, the catalytic performances of the free catalytic salts (without avidin) are similar to, if not better than, those of the biohybrid catalysts. While it seems that avidin does not change the performances of these catalysts, there is no evidence of particular new interactions between the

Scheme 1. Preparation of the Anions



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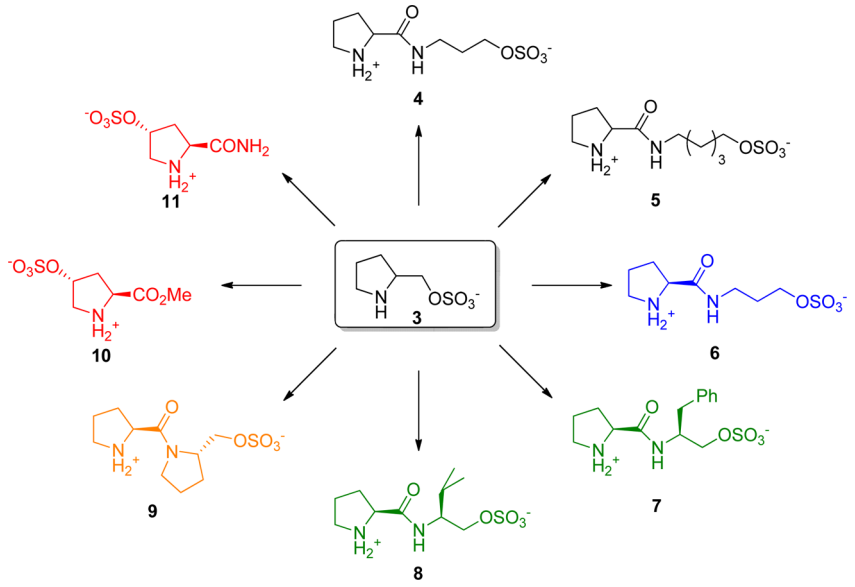
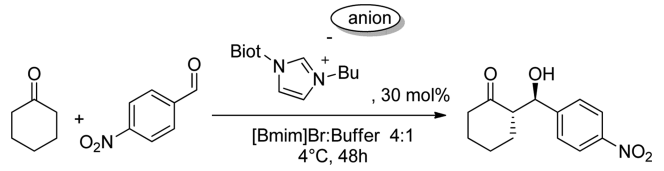


Figure 3. The different zwitterions used as precursors for the biotinylated catalysts.

Table 1. Anion Screening



		with avidin			without avidin[b]		
entry	anion	conversion (%)[a]	dr (*syn*/*anti*)[a]	ee (*anti*) (%)[a]	conversion (%)[a]	dr (*syn*/*anti*)[a]	ee (*anti*) (%)[a]
1	**3**	94	32/68	70	98	36/64	rac
2	**4**	63	32/68	54	>99	35/65	rac
3	**5**	93	31/69	53	94	36/64	rac
4	**6**	76	33/67	70	>99	35/65	69
5	**7**	94	23/77	80	>99	24/76	83
6	**8**	75	27/73	76	>99	27/73	81
7	**9**	62	30/70	71	58	54/46	83
8	**10**	98	36/64	50	89	35/65	53
9	**11**	93	35/65	51	98	20/80	82

[a]Determined by chiral HPLC. [b][substrate] = 1 M.

side chains added to the L-proline and avidin for the complexed catalysts.

(d) Lowering the number of possible conformations of the anion inside avidin, as in the case of anion **9**, based on a Pro-Pro scaffold (entry 7), did not lead to a better stereocontrol of the reaction, in comparison to anion **3**. Adding steric hindrance to the anion seems to lower the reactivity of the whole catalyst.

(e) We previously reported the use of a 1-butyl-3-methylimidazolium salt bearing anions **10** and **11** as catalysts for the aldol reaction.[17] The use of *trans*-4-hydroxy-L-proline derivatives in combination with the biotinylated imidazolium cation allowed the displacement of the sulfate charge closer to the amine site, in comparison to compounds **4**–**9**. However, the catalytic performances of the corresponding assembled catalysts were still lower than those obtained with **3**. Also, the introduction of a hydrogen-bonding group on the anion **11** did not enhance in any way the performance of the corresponding **11**·**Av** biohybrid catalyst.

In light of these results, it seems that no strict rule can be applied toward the design of improved anions for our system, even though chiral anions perform better than racemic ones. We performed deeper investigations with the anion **7**, in order to get a better understanding of the possible match–mismatch cases observed when anion **6** was used. In this case, all four diastereoisomers of compound **7** were prepared (Figure 4) and the corresponding biohybrid catalysts assembled.

Once again, their catalytic activity was assessed both with and without avidin in the aldol reaction (Table 2). When catalytic tests were carried out without avidin, the corresponding biotinylated catalysts afforded the aldol products with excellent conversion and good selectivities. The use of **7**·**Av** and **14**·**Av** gave the best results in terms of selectivity, each enantiomer of the anion affording each enantiomer of the aldol product with the same ee value. Using **12**·**Av** and **13**·**Av** led to lower ee values but once again expectedly led each to each enantiomer of the aldol product. However, their behavior inside avidin is quite

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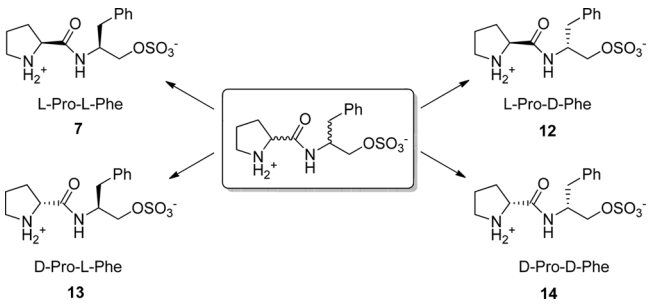


Figure 4. Diastereomers of anion **7**.

different. A trend emerges when looking at the results given in Table 2.

The best results were achieved both in terms of selectivity and conversion when **7·Av** was used, but these results were similar to those obtained without avidin. Nevertheless, the influence of avidin was more pronounced when biohybrid catalysts **12·Av**–**14·Av** were used. Surprinsingly, better results were achieved when only natural amino alcohols were used. The use of L-Proline as the first amino acid led to better results in terms of conversion and enantioselectvity with L-phenylalaninol, in comparison to those obtained when D-phenylalaninol was used. Replacing L-proline by D-proline drastically diminished the performances of the biohybrid catalyst, as ee values decreased respectively to 6% and −9% (Table 2, entries 3 and 4), indicating that the chiral center closest to the amine site is the most important for the stereocontrol of the reaction. The drop of selectivity observed for D-proline and D-phenylalaninol inside avidin may be the result of the competition between the chiral induction of the avidin cavity and the intrinsic chirality of the anion. This is a significant evidence of a match−mismatch case, as the corresponding catalyst in the absence of avidin afforded a better selectivity of the reaction. The addition of a side chain also seems beneficial for the stereocontrol of the reaction, and a fine-tuning process for the design of new catalytic anions using other natural amino alcohols is currently undergoing study in our group.

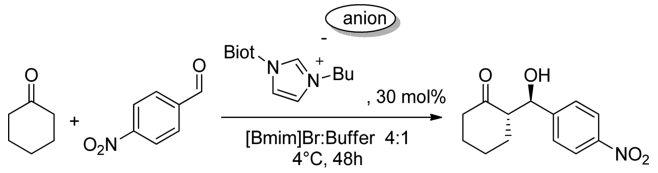
We also speculated that the use of a biohybrid catalyst in an ionic liquid/aqueous mixture would be an asset in terms of substrate tolerance, as the anion possesses a greater freedom of movement inside avidin's cavity, in comparison to a covalently biotinylated amine catalyst. The biohybrid catalyst **7·Av** was used for substrate screening (Table 3). We were pleased to observe that our biohybrid catalyst tolerates several ketones and aldehydes. The use of a bulky aldehyde (entry 5) led to good results in terms of conversion and selectivity, despite a longer reaction time. Fluorous aldehydes were also tested and afforded the corresponding aldol products with excellent conversions and selectivities (entries 6 and 7). 2-thiocarboxaldehyde, however, displayed poor reactivity in spite of a good selectivity.

An intramolecular aldol reaction was also performed using 2-(2-oxopropoxy)benzaldehyde (Table 3, entry 9). The dihydrobenzofuranol resulting from the intramolecular aldol is a common motif found in several natural coumarins used for the treatment of skin diseases, such as vitiligo and psoriasis. Enders et al. used keto aldehyde derivatives (entry 9) to study this type of intramolecular aldol reaction, using L-proline as catalyst, and applied this procedure to the total synthesis of smyrindiol.[18,19] In the presence of **7·Av**, as well as **7** without avidin, we were surprised to observe the exclusive formation of the condensation product despite the absence of dehydration conditions, the formation of the aromatic benzofuranol being quite favored.

■ CONCLUSION

The preparation and catalytic studies of several anions in combination with a biotinylated imidazolium salt assessed the influence of the anion structure on the activity of biohybrid catalysts obtained with avidin. Even though the observed effects are still not completely understood, it has been shown that the intrinsic chirality of the anion has an important influence on the catalytic properties of the biohybrid species assembled with avidin. A match−mismatch case was also identified, supporting the hypothesis of cooperativity between avidin and the catalytic salt itself, in terms of stereocontrol of the aldol reaction. The substrate tolerance of the biohybrid catalyst was also put to use through the use of several ketones/aldehydes in the aldol reaction. Further optimization of the system could likely be achieved through molecular modeling and docking studies to identify the residues involved in the catalytic process. By saturation mutagenesis and directed evolution, fine tuning might also be achieved to further raise the cooperativity between the catalyst and the avidin. The advantage of these biohybrid system is that the catalyst and the protein could be optimized independently, followed by screening all combinations in a matrix format, if desired. The synthesis of other anions with different catalytic properties may broaden the scope of other possible reactions.

Table 2. Influence of the Chirality of the Anion



		with avidin			without avidin[b]		
entry	anion	conversion (%)[a]	dr (*syn*/*anti*)[a]	ee (*anti*) (%)[a]	conversion (%)[a]	dr (*syn*/*anti*)[a]	ee (*anti*) (%)[a]
1	**7** (L-Pro-L-Phe)	94	23/77	80	>99	24/76	83
2	**12** (L-Pro-D-Phe)	86	28/72	63	>99	27/73	62[c]
3	**13** (D-Pro-L-Phe)	87	37/63	6	89	29/71	−63
4	**14** (D-Pro-D-Phe)	96	25/75	−9[c]	87	30/70	−79[c]

[a]Determined by chiral HPLC. [b][substrate] = 1 M. [c]The enantiomer of the aldol product was obtained.

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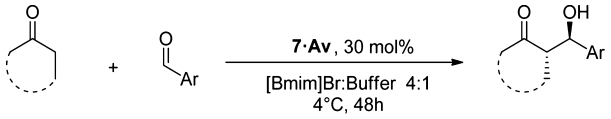
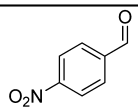
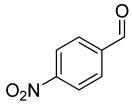
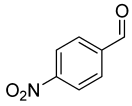
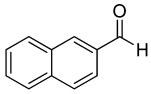
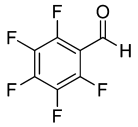
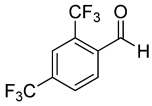
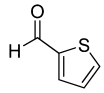
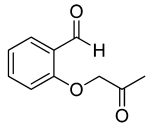
Table 3. Substrate Scope



Entry	Ketone	Aldehyde	Conversion (%)[a]	*dr* (*syn/anti*)[a]	*ee* (anti)[a]
1			94	23/77	80%
2			›99	64/36	34%
3			91	-	rac.
4[b]			10	24/76	88%
5			93	6/94	91%
6			›99	›1/99	84%
7			10	36/64	81%.
9				›99% (Condensation product)	

[a]Determined by chiral HPLC. [b]The reaction mixture was stirred for 5 days.

EXPERIMENTAL SECTION

Materials. All organic compounds were obtained commercially and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on 400, 300, 100, and 75 MHz spectrometers, in the indicated solvent. Chemical shifts are reported in ppm with internal reference to TMS, and *J* values are given in hertz. All HPLC analyses were done using Chiralpak AD-H or OD columns, and absolute configurations of the aldol products were based on previously reported literature data.[17] HRMS/ESI measurements were done on a Quantum TSQ Ultra instruments.

Boc-proline was obtained following the procedure described by Azizi et al.[20]

Representative Procedure for the Peptidic Coupling between Proline and the Corresponding Amino Alcohol. The **1.*X*** family of compounds refers to the Boc precursors of the corresponding *X* zwitterion, as they are mentioned in the paper.

Boc-proline (1 equiv) and *N*-hydroxysuccinimide (1 equiv) were dissolved in acetonitrile (1 mL/mmol of proline). Dicyclohexylcarbodiimide (1 equiv) was then added to the reaction mixture, and the formation of a precipitate (DCU) was observed after 10 min. The corresponding amino alcohol was then added to the reaction vessel, and the suspension was refluxed overnight. Upon completion, the reaction mixture was cooled to room temperature and filtered to remove DCU. Methylene chloride was added to the filtrate, and the organic phase was washed with water, dried with magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, affording the desired compound

*tert-Butyl 2-((3-Hydroxypropyl)carbamoyl)pyrrolidine-1-carboxylate (**1.4** and **1.6**).* Yields: white foam, 344.1 mg, 68% (compound **1.4**); white foam, 308.6 mg, 61% (compound **1.6**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.56−7.16 (m, 1 H), 4.24 (br s, 1 H), 3.16−3.71 (m, 7 H), 1.78−2.36 (m, 4 H), 1.62−1.75 (m, 2 H), 1.44 ppm (s, 9 H). ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz; mixture of rotamers): δ 173.9, 173.4, 155.6, 154.6, 80.5, 61.2, 60.1, 59.2, 47.1, 36.1 (br s), 32.3, 31.2 (br s), 28.4, 24.6, 23.7 ppm. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{13}H_{25}N_2O_4$, 273.1809; found, 273.1802.

*tert-Butyl 2-((5-Hydroxypentyl)carbamoyl)pyrrolidine-1-carboxylate (**1.5**).* Yield: pale yellow foam, 362.4 mg, 65%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.02−7.02 (m, 1 H), 4.26 (br s, 1 H), 3.65 (t, *J* = 6.0 Hz, 2 H), 3.07−3.51 (m, 4 H), 1.89 (br s, 2 H), 1.77 (br s, 3 H), 1.36−1.64 ppm (m, 15 H). ^{13}C{^{1}H} NMR ($CDCl_3$, 75 MHz; mixture of

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rotamers): δ 173.8, 173.5, 155.7, 154.7, 80.7, 61.2, 60.1, 59.2, 47.1, 36.2 (br s), 32.2, 31.1 (br s), 28.4, 24.6, 23.6 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{15}H_{29}N_2O_4$, 301.2122; found, 301.2116.

*tert-Butyl 2-((1-Hydroxy-3-phenylpropan-2-yl)carbamoyl)-pyrrolidine-1-carboxylate (**1.7** and **1.14**).* Yields: white foam, 394.9 mg, 61% (compound **1.7**); white foam, 284.8 mg, 44% (compound **1.14**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.13−7.33 (m, 5 H), 6.24−6.80 (m, 1 H), 4.19 (br s, 2 H), 3.49−3.74 (m, 2 H), 3.17−3.46 (m, 3 H), 2.66−3.02 (m, 2 H), 1.55−2.24 (m, 4 H), 1.44 ppm (br s, 9 H). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz; mixture of rotamers): δ 173, 172.1, 155.7, 154.6, 137.7 (br s), 129.1, 128.5, 126.5, 80.7, 64.3, 63.8, 61.2, 60.5, 52.7, 52.4, 47. (br s), 37, 33.9, 30.9 (br s), 28.7, 28.3, 24.3, 23.3 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{19}H_{29}N_2O_4$, 349.2122; found, 349.2120.

*tert-Butyl 2-((1-Hydroxy-3-phenylpropan-2-yl)carbamoyl)-pyrrolidine-1-carboxylate (**1.12** and **1.13**).* Yields: white foam, 439.7 mg, 68% (compound **1.12**); white foam, 405.3 mg, 63% (compound **1.13**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.12−7.36 (m, 5 H), 6.72 (br s, 1 H), 4.08−4.23 (m, 2 H), 3.22−3.76 (m, 5 H), 2.88 (d, *J* = 7.7 Hz, 2 H), 1.69−2.21 (m, 4 H), 1.44 ppm (br s, 9 H)., $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz; mixture of rotamers): δ 173.2, 172.7, 155.6, 154.6, 137.9, 129.2, 128.5, 126.5, 80.6, 63.4, 61.2, 60.3, 53.1, 52.7, 47.1, 46.8, 36.9, 31.1, 28.9, 28.4, 24.6, 23.6 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{19}H_{29}N_2O_4$, 349.2122; found, 349.2125.

*(S)-tert-Butyl 2-(((S)-1-Hydroxy-3-methylbutan-2-yl)carbamoyl)-pyrrolidine-1-carboxylate (**1.8**).* Yield: white foam, 312.2 mg, 56%. Spectroscopic data were in accordance with literature data.[21]

Preparation of (*S*)-*tert*-Butyl 2-((*S*)-2-(Hydroxymethyl)-pyrrolidine-1-carbonyl)pyrrolidine-1-carboxylate (1.9). Under a nitrogen atmosphere, Boc-protected L-proline (1 g, 4.646 mmol, 1.05 equiv) and L-proline methyl ester hydrochloride[22] (1 equiv) were suspended in 25 mL of dry CH_2Cl_2, and DIPEA (3 equiv) was added. The limpid solution was cooled to 0 °C, and a solution of DCC (1.2 equiv) in dry DCM (5 mL) was slowly added. The solution was stirred for 30 min at 0 °C and then overnight at room temperature. After completion of the reaction, the DCU was filtered and the organic phase was washed with water, dried with magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 99/1 then 95/5) to afford the desired Pro-Pro derivative **1.9-A** as a sticky foam (1.04 g, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.34−4.63 (m, 2 H), 3.77 (d, *J* = 9.5 Hz, 1 H), 3.67−3.72 (m, 3 H), 3.33−3.66 (m, 3 H), 1.77−2.25 (m, 8 H), 1.33−1.49 ppm (m, 9 H), $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz; mixture of rotamers): δ 172.9, 172.6, 171.6, 171.1, 154.6, 153.7, 79.5, 79.4, 58.7 (d), 57.8, 57.7, 52.2, 52.0, 46.9,46.7, 46,5 (d), 30, 29.0, 28.8, 28.7, 28.5, 28.4, 25 (d), 24.1, 23.6 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{16}H_{27}N_2O_5$, 327.1914; found, 327.1914. Compound **1.9** was then prepared following a procedure reported by De Souza et al.[23] In a three-neck flask, compound **1.9-A** (659.2 mg) was dissolved in THF (10 mL), and $NaBH_4$ (6 equiv) was added. The mixture was stirred at reflux for 15 min, and then methanol (6 mL) was slowly added using an addition funnel over 30 min, inducing effervescence in the flask. The reaction was stirred at reflux for 1 h more. After TLC completion, excess hydride was slowly quench edusing H_2O and the mixture was extracted three times with CH_2Cl_2. The combined organic phases were dried over magnesium sulfate, filtered, and evaporated in vacuo to afford the desired product as a pale yellow foam (92%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.80−5.04 (m, 1 H), 4.02−4.55 (m, 2 H), 3.34−3.91 (m, 6 H), 1.50−2.31 (m, 8 H), 1.32−1.48 ppm (m, 9 H). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz; mixture of rotamers): δ 174.2, 173.8, 171.6, 154.9, 154.2, 153.3, 79.8, 79.2, 67.2, 67.1, 66.2, 61.2, 60.8, 59.3, 57.6, 57.2, 47.2, 46.9, 46.5, 46.3, 45.3, 30.3, 29.5 (d), 28.9, 28.1, 28, 27.6, 27.4, 24.3, 24.2, 23.9, 23.3, 21.9 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{15}H_{27}N_2O_4$, 299.1965; found, 299.1963. NMR of this compound in dmso-d_6 was carried out at different temperatures to confirm the presence of rotamers (see the Supporting Information).

Representative Procedure for the Preparation of Zwitterions 4−9 and 13−15. Under nitrogen, the corresponding alcohol **1.*X*** was dissolved in dry dichloromethane (3 mL/mmol) and cooled to 0 °C. Chlorosulfonic acid (1 equiv) was added dropwise to the reaction mixture, and the reaction mixture was stirred for an additional 2 h at room temperature. After the reaction, the solvent was discarded and purification by achieved by flash chromatography on silica gel (CH_2Cl_2/MeOH 8/2), affording the desired compound.

*3-(Pyrrolidin-1-ium-2-carboxamido)propyl Sulfate (**4** and **6**).* Yields: white sticky foam, 173.8 mg, 53% (compound **4**); white sticky foam, 147.5 mg, 62% (compound **6**). ^{1}H NMR (D_2O, 400 MHz): δ 4.26 (dd, *J* = 8.3, 6.6 Hz, 1 H), 4.02 (t, *J* = 6.0 Hz, 2 H), 3.23−3.41 (m, 4 H), 2.35 (d, *J* = 8.4 Hz, 1 H), 1.92−2.04 (m, 3 H), 1.80−1.89 ppm (m, 2 H). $^{13}C\{^1H\}$ NMR (D_2O, 75 MHz): δ 169.5, 66.7, 59.8, 46.4, 36.5, 29.6, 27.7, 23.8 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_8H_{16}N_2O_5S$, 253.0853; found, 253.0855.

*5-(Pyrrolidin-1-ium-2-carboxamido)pentyl Sulfate (**5**).* Yield: pale yellow sticky foam, 147.4 mg, 42%. ^{1}H NMR (D_2O, 400 MHz): δ 4.25 (t, *J* = 7.2 Hz, 1 H), 3.97 (t, *J* = 6.1 Hz, 2 H), 3.08−3.42 (m, 4 H), 2.30−2.43 (m, 1 H), 1.89−2.02 (m, 3 H), 1.61 (quin, *J* = 6.7 Hz, 2 H), 1.49 (quin, *J* = 7.0 Hz, 2 H), 1.33 ppm (quin, *J* = 7.5 Hz, 2 H). $^{13}C\{^1H\}$ NMR (D_2O, 75 MHz): δ 168.9, 68.8, 59.4, 46.1, 39, 29.4, 27.5, 27.1, 23.4, 21.7 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{10}H_{21}N_2O_5S$, 281.1166; found, 281.1160.

*3-Phenyl-2-(pyrrolidin-1-ium-2-carboxamido)propyl Sulfate (**7** and **14**).* Yields: white solid, 381.9 mg, 92% (compound **7**); white solid, 268.6 mg, 69% (compound **14**). These compounds can be purified by recrystallization in ethanol. ^{1}H NMR (D_2O, 400 MHz): δ 7.17−7.38 (m, 5 H), 4.27−4.38 (m, 1 H), 4.11−4.20 (m, 1 H), 4.03−4.10 (m, 1 H), 3.90−4.01 (m, 1 H), 3.17−3.39 (m, 2 H), 2.88−2.98 (m, 1 H), 2.68−2.79 (m, 1 H), 2.24−2.38 (m, 1 H), 1.94 ppm (d, *J* = 6.4 Hz, 3 H). $^{13}C\{^1H\}$ NMR (D_2O, 75 MHz): δ 170, 138.4, 130.1, 129.5, 127.7, 70, 60.4, 51.7, 473, 36.5, 30.7, 24.4 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{14}H_{20}N_2O_5S$, 329.1166; found, 329.1173.

*3-Phenyl-2-(pyrrolidin-1-ium-2-carboxamido)propyl Sulfate (**12** and **13**).* Yields: white solid, 281 mg, 62% (compound **12**); white solid, 221.8 mg, 68% (compound **13**). These compounds can be purified by trituration in cold ethanol. ^{1}H NMR (D_2O, 400 MHz): δ 7.13−7.38 (m, 5 H), 4.40 (m, 1 H), 4.07−4.23 (m, 2 H), 3.96−4.04 (m, 1 H), 3.18 (t, *J* = 7.1 Hz, 2 H), 2.99 (dd, *J* = 14.2, 4.1 Hz, 1 H), 2.67 (m, 1 H), 2.09 (m, 1 H), 1.80 (m, 1 H), 1.58 (m, 1 H), 1.34 ppm (m, 1 H). $^{13}C\{^1H\}$ NMR (D_2O, 75 MHz): δ 169.9, 138.2, 130.1, 129.4, 127.6, 70.4, 60.4, 51.3, 47.1, 36.9, 30.6, 24.2 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{14}H_{20}N_2O_5S$, 329.1166; found, 329.1173.

*(S)-3-Methyl-2-((S)-pyrrolidin-1-ium-2-carboxamido)butyl Sulfate (**8**).* Yield: white foam, 218.8 mg, 42%; ^{1}H NMR (D_2O, 400 MHz): δ 4.29 (dd, *J* = 8.3, 6.7 Hz, 1 H), 4.02−4.10 (m, 1 H), 3.94−4.01 (m, 1 H), 3.79 (td, *J* = 7.3, 3.6 Hz, 1 H), 3.21−3.42 (m, 2 H), 2.29−2.41 (m, 1 H), 1.90−2.09 (m, 3 H), 1.74−1.85 (m, 1 H), 0.86 ppm (dd, *J* = 16.8, 6.7 Hz, 6 H). $^{13}C\{^1H\}$ NMR (D_2O, 75 MHz): δ 170.1, 69.5, 59.8, 55, 46.5, 30.1, 28.5, 23.7, 18.5, 18 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{10}H_{20}N_2O_5S$, 281.1166; found, 281.1174.

*((S)-1-((S)-Pyrrolidin-1-ium-2-carbonyl)pyrrolidin-2-yl)methyl Sulfate (**9**).* Yield: white solid, 134 mg, 28%; ^{1}H NMR (D_2O, 500 MHz; mixture of rotamers): δ 5.03−5.32 (m, 1 H), 4.80−4.88 (m, 1 H), 4.75 (dd, *J* = 10.3, 4.8 Hz, 1 H), 4.46−4.59 (m, 1 H), 4.08−4.15 (m, 1 H), 3.97−4.05 (m, 2 H), 3.92 (tt, *J* = 10.8, 7.0 Hz, 1 H), 2.97−3.10 (m, 1 H), 2.37−2.66 ppm (m, 7 H). $^{13}C\{^1H\}$ NMR (D_2O, 100 MHz; mixture of rotamers): δ 168.9, 168.4, 69.6, 68.1, 60.1, 59.9, 57.6, 57.4, 48.1, 47.6, 47.4, 46.8, 29.4, 29.1, 18.2, 27.1, 24.5, 24.3(9), 24.3(6), 21.6 ppm. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_5H_9N_2O_5S^-$, 209.02377; found, 209.02386.

Representative Procedure for the Preparation of Biotinylated Salts Biot-4−Biot-11. The **Biot-*X*** family of compounds refers to the corresponding biotinylated imidazolium salts bearing the anion ***X***. These compounds are NOT designated as such in the paper, to avoid confusion with the biohybrid catalysts.

The biotinylated imidazolium bromide salt precursor was synthesized following our reported procedure. The preparation of the catalytic biotinylated imidazolium salts is based on our published procedure, with minor modifications.

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The biotinylated imidazolium bromide salt (50 mg, 0.102 mmol) was dissolved in MeOH (2 mL) and passed through an IRA-400 HO form exchange resin (1.5 g). The resin was then washed with MeOH (2 × 3 mL), and zwitterion **6** (1.05 equiv) was added to the reaction vessel. The mixture was stirred overnight at room temperature. The methanol and resulting water were removed under reduced pressure, and the residue was dissolved in 1 mL of CH_3CN/MeOH (95/5) and cooled to −8 °C for 3 h. The resulting solid was filtered, and the filtrate was evaporated under reduced pressure and dried under vacuum to give the desired product as a yellow foam.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4-yl)pentanamido)propyl)-1H-imidazol-3-ium 3-(Pyrrolidine-2-carboxamido)propyl Sulfate (**Biot-4** and **Biot-6**).* Yields: 47.8 mg, 71% (compound **Biot-4**); 51.8 mg, 77% (compound **Biot-6**). ^{1}H NMR (D_2O, 400 MHz): δ 7.44 (s, 2 H), 4.53 (dd, J = 7.6, 4.9 Hz, 1 H), 4.34 (dd, J = 7.8, 4.5 Hz, 1 H), 4.14 (dt, J = 13.8, 7.0 Hz, 4 H), 4.01 (t, J = 6.0 Hz, 2 H), 3.73 (d, J = 6.6 Hz, 1 H), 3.22−3.31 (m, 2 H), 3.12−3.20 (m, 2 H), 2.83−3.02 (m, 3 H), 2.68 (d, J = 13.0 Hz, 1 H), 1.99−2.25 (m, 5 H), 1.16−1.88 (m, 16 H), 0.85 ppm (t, J = 7.4 Hz, 3 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 176.9, 175.8, 165.3, 122.5, 122.3, 66.7, 62.1, 60.2, 60.1, 55.5, 49.4, 47, 46.4, 39.7, 36.1, 35.8, 35.4, 31.2, 30.3, 28.9, 28, 27.9, 27.7, 25.1, 25, 18,8, 12.6 ppm. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S^+$, 408.2428; found, 408.2436; m/z [M*]$^-$ calcd for $C_8H_{15}N_2O_5S^-$, 251.0707; found, 251.0713.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4-yl)pentanamido)propyl)-1H-imidazol-3-ium 5-(Pyrrolidine-2-carboxamido)pentyl Sulfate (**Biot-5**).* Yield: 48.4 mg, 69%. ^{1}H NMR (D_2O, 400 MHz): δ 7.44 (s, 2 H), 4.46−4.56 (m, 1 H), 4.33 (dd, J = 7.5, 4.4 Hz, 1 H), 4.14 (dt, J = 14.0, 7.0 Hz, 4 H), 3.97 (t, J = 6.1 Hz, 2 H), 3.77 (d, J = 6.6 Hz, 1 H), 3.07−3.32 (m, 5 H), 2.85−3.06 (m, 3 H), 2.67 (d, J = 13.0 Hz, 1 H), 2.08−2.23 (m, 3 H), 2.03 (m, 2 H), 1.42−1.83 (m, 13 H), 1.16−1.37 (m, 6 H), 0.84 ppm (t, J = 7.3 Hz, 3 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 176.9, 174.7, 165.3, 122.5, 122.3, 69.1, 621, 60.3, 60.1, 55.5, 49.4, 47, 46.4, 39.7, 39.1, 35.9, 35.4, 31.2, 30.4, 28.9, 28, 28, 27.8, 27.7, 25.1, 24.9, 22.3, 18.8, 12.7 ppm. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S$, 409.2506; found, 409.2489; m/z [M*]$^-$ calcd for $C_{10}H_{19}N_2O_5S^-$, 279.1027; found, 279.1027.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4-yl)pentanamido)propyl)-1H-imidazol-3-ium 3-Phenyl-2-(pyrrolidine-2-carboxamido)propyl Sulfate (**Biot-7** and **Biot-14**).* Yields: 73.5 mg, 98% (compound **Biot-7**); 53.3 mg, 71% (compound **Biot-14**). ^{1}H NMR (D_2O, 400 MHz): δ 7.41 (s, 2 H), 7.13−7.33 (m, 5 H), 4.43−4.53 (m, 1 H), 4.22−4.35 (m, 2 H), 4.05−4.17 (m, 4 H), 3.89−4.05 (m, 2 H), 3.70 (dd, J = 8.2, 6.3 Hz, 1 H), 3.09−3.26 (m, 3 H), 2.81−2.96 (m, 4 H), 2.59−2.78 (m, 2 H), 2.15 (t, J = 7.2 Hz, 2 H), 1.95−2.10 (m, 3 H), 1.39−1.81 (m, 9 H), 1.16−1.38 (m, 4 H), 0.83 ppm (t, J = 7.3 Hz, 3 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 176.8, 174.4, 165.2, 137.6, 129.3, 128.6, 126.8, 122.5, 122.3, 69.2, 60.2, 59.9, 55.5, 50.3, 49.4, 47, 46.4, 39.7, 36.1, 35.8, 34.4, 31.2, 30.3, 28.9, 28, 27.7, 25.1, 24.7, 19.9, 12.7 ppm. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S^+$, 408.2428; found, 408.2438; m/z [M*]$^-$ calcd for $C_{14}H_{19}N_2O_5S$, 327.1020; found, 327.1029.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4-yl)pentanamido)propyl)-1H-imidazol-3-ium 3-Phenyl-2-(pyrrolidine-2-carboxamido)propyl Sulfate (**Biot-12** and **Biot-13**).* Yields: 63.3 mg, 91% (compound **Biot-12**); 61.5 mg, 82% (compound **Biot-13**). ^{1}H NMR (D_2O, 400 MHz): δ 7.41 (s, 2 H), 7.15−7.30 (m, 5 H), 4.49 (s, 1 H), 4.25−4.36 (m, 2 H), 3.94−4.17 (m, 6 H), 3.66−3.74 (m, 1 H), 3.17−3.27 (m, 1 H), 3.10−3.16 (m, 2 H), 2.81−2.97 (m, 4 H), 2.60−2.71 (m, 2 H), 2.16 (t, J = 7.2 Hz, 2 H), 2.00 (dd, J = 14.2, 7.4 Hz, 3 H), 1.70−1.81 (m, 2 H), 1.41−1.69 (m, 6 H), 1.15−1.39 (m, 5 H), 0.83 ppm (t, J = 7.4 Hz, 3 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 176.5, 173.5, 164.9, 137.2, 129, 128.2, 126.4, 122.1, 121.9, 69.1, 61.7, 59.9, 59.4, 55.1, 49.8, 49, 46.6, 45.8, 39.3, 35.8, 35.4, 35, 30.8, 28.5, 27.6, 27.3, 24.7, 24.1, 18.4, 12.3 ppm. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S^+$, 408.2428; found, 408.2432; m/z [M*]$^-$ calcd for $C_{14}H_{19}N_2O_5S^-$, 327.1020; found, 327.1024.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4-yl)pentanamido)propyl)-1H-imidazol-3-ium (S)-3-Methyl-2-((S)-pyrrolidine-2-carboxamido)butyl Sulfate (**Biot-8**).* Yield: 68.7 mg, 98%. ^{1}H NMR (D_2O, 400 MHz): δ 7.44 (s, 2H), 4.52 (br s, 1 H), 4.33 (br s, 1 H), 4.14 (dt, J = 13.2, 6.6 Hz, 4 H), 3.97−4.03 (m, 2 H), 3.77 (d, J = 5.7 Hz, 2 H), 3.11−3.30 (m, 3 H), 2.85−3.05 (m, 3 H), 2.67 (d, J = 13.0 Hz, 1 H), 1.96−2.24 (m, 5 H), 1.18−1.85 (m, 14 H), 0.72−0.93 ppm (m, 9 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 176.8, 175.4, 165.3, 122.5, 122.3, 68.5, 62.11, 60.25, 60.1, 5..5, 54.4, 49.4, 47, 46.5, 39.7, 35.8, 35.4, 31.2, 30.63, 28.9, 28.6, 28, 27.7, 25.1, 24.9, 18.8, 18.6, 17.9, 12.7 ppm. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S^+$, 408.2428; found, 408.2435; m/z [M*]$^-$ calcd for $C_{10}H_{19}N_2O_5S^-$, 279.1020; found, 279.1028.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4-yl)pentanamido)propyl)-1H-imidazol-3-ium ((S)-1-((S)-Pyrrolidine-2-carbonyl)pyrrolidin-2-yl)methyl Sulfate (**Biot-9**).* Yield: 231 mg, 33%. ^{1}H NMR (D_2O, 400 MHz): δ 7.44 (s, 2 H), 4.53 (dd, J = 7.8, 4.9 Hz, 1 H), 4.06−4.37 (m, 7 H), 3.95 (dd, J = 9.6, 2.8 Hz, 1 H), 3.37−3.58 (m, 2 H), 2.86−3.30 (m, 6 H), 2.68 (d, J = 13.0 Hz, 1 H), 1.13−2.39 (m, 23 H), 0.84 ppm (t, J = 7.3 Hz, 3 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 177, 171.3, 165.3, 122.5, 122.3, 68.9, 67.5, 32.1, 60.2, 28,9, 28.7, 26.7, 56.4, 55.4, 55.4, 49.4, 47.4, 47, 46.8, 46.6, 46.1, 39.7, 35.8, 35.4, 31.2, 29.4, 29.2, 28.9, 28, 27.7, 27.5, 26.5, 25, 24.8, 24.7, 23.8, 21, 18.8, 12.6 ppm. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S^+$, 408.2428; found, 408.2436; m/z [M*]$^-$ calcd for $C_{10}H_{17}N_2O_5S^-$, 277.0864; found, 277.0869.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4-yl)pentanamido)propyl)-1H-imidazol-3-ium (3R,5S)-5-(Methoxycarbonyl)pyrrolidin-3-yl Sulfate (**Biot-10**).* Yield: 58.7 mg, 91%. ^{1}H NMR (D_2O, 400 MHz): δ 8.71 (s, 1 H), 7.44 (s, 2 H), 4.99 (br s, 1 H), 4.48−4.56 (m, 1 H), 4.30−4.37 (m, 1 H), 4.08−4.25 (m, 5 H), 3.71 (s, 4 H), 3.08−3.33 (m, 5 H), 2.86−2.94 (m, 1 H), 2.68 (d, J = 12.8 Hz, 1 H), 2.46−2.55 (m, 1 H), 2.18 (t, J = 7.3 Hz, 3 H), 2.04 (t, J = 6.8 Hz, 2 H), 1.16−1.83 (m, 9 H), 0.84 ppm (t, J = 7.4 Hz, 3 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 176.9, 174, 165.3, 135.3, 122.5, 12.3, 79.3, 62.1, 60.3, 58, 55.5, 53.1, 51.9, 49.4,47.1, 39.7, 35.9, 35.8, 35.4, 32.2, 28.9, 28, 27.7, 25.1, 18.8, 12.6 ppm. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S^+$, 408.2428; found, 408.2240; m/z [M*]$^-$ calcd for $C_6H_{10}NO_6S^-$, 224.0234; found, 224.0234.

*3-Butyl-1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]-imidazol-4 yl)pentanamido)propyl)-1H-imidazol-3-ium (3R,5S)-5-Carbamoylpyrrolidin-3-yl Sulfate (**Biot-11**).* Yield: 55.5 mg, 88%. ^{1}H NMR (D_2O, 400 MHz): δ 8.72 (s, 1 H), 7.44 (s, 2 H), 4.95 (br s, 1 H), 4.50−4.56 (m, 1 H), 4.31−4.37 (m, 1 H), 4.14 (dt, J = 13.9, 7.1 Hz, 4 H), 3.94 (s, 1 H), 3.13−3.30 (m, 4 H), 3.10 (d, J = 4.0 Hz, 1 H), 2.89 (d, J = 5.0 Hz, 1 H), 2.68 (d, J = 13.0 Hz, 1 H), 2.40−2.48 (m, 1 H), 2.18 (t, J = 7.2 Hz, 2 H), 1.95−2.09 (m, 3 H), 1.72−1.83 (m, 2 H), 1.43−1.71 (m, 4 H), 1.24 (d, J = 7.7 Hz, 4 H), 0.85 ppm (t, J = 7.4 Hz, 3 H). ^{13}C{^{1}H} NMR (D_2O, 75 MHz): δ 178.4, 177, 165.3, 135.3, 122.5, 122.3, 80.8, 62.1, 62.3, 58.5, 55.4, 52.3, 49.4, 47, 39.7, 7.1, 35.8, 35.4, 31.22, 28.9, 28, 27.7, 25.1, 18.8, 12.6. HRMS (ESI): m/z [M*]$^+$ calcd for $C_{20}H_{34}N_5O_2S^+$, 408.2428; found, 408.2436; m/z [M*]$^-$ calcd for $C_5H_9N_2O_5S^-$, 209.0238; found, 209.0239.

Representative Procedure for the Aldol Reaction. The aldol reaction is carried in 100 μL of a 8/2 solution of [Bmim]Br and Tris buffer solution. Stock solutions of starting materials and catalysts were prepared: $\mathbf{S_1}$ refers to a 0.2 M solution of aldehyde in [Bmim]Br, $\mathbf{S_2}$ refers to a 2 M solution of cyclohexanone in [Bmim]Br, and $\mathbf{S_3}$ refers to a 0.03 M solution catalyst in Tris buffer solution (pH 3). In a vial, 12 mg of avidin (13.8 U/mg, binds 0.678 μmol of biotynilated catalyst, 1.13 equiv compared to catalyst) was suspended in 60 μL of [Bmim]Br. A 10 μL portion of $\mathbf{S_2}$ (20 μmol, 10 equiv) and 20 μL of $\mathbf{S_3}$ (0.6 μmol, 30 mol %) were added. Avidin was dissolved in the reaction medium, and the reaction vial was stirred for 15 min at room temperature to allow the preformation of the catalytic species. A 10 μL portion of $\mathbf{S_1}$ (2 μmol, 1 equiv) was added, and the reaction mixture was stirred for 48 h at 4 °C. Ether (0.5 mL) was then added, and the vial was vortexed for 1 min. The ethereal phase was analyzed by chiral HPLC (ChiralPak AD-H column, hexanes/IPA 95/5, 0.5 mL/min, λ 254 nm): t_R(*anti* isomer) = 47.10 (minor), 63.90 (major), t_R(*syn* isomer) = 32.14 (minor), 42.81 min (major).

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■ ASSOCIATED CONTENT

Supporting Information

Figures giving ^{1}H and ^{13}C NMR spectra and HPLC traces. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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